

REMARKS

Applicants' attorney acknowledges with appreciation the interview courteously extended by Examiner, Afremova and Primary Examiner, Irene Marx on 10/23/03. During the interview, it was agreed that applicants would submit amendments and/or arguments drawn to the step of "suspending" the cells in the absence of sulfur in the sulfur-free medium and sealing from atmospheric oxygen until "conditions" became anaerobic; indicate the use of illumination conditions in each step when it is required to sustain hydrogen production; and clarify the step of "controlling".

The Official Action and the cited references have been carefully reviewed. The review indicates that the claims, especially as amended, recite patentable subject matter.

Reconsideration and allowance are therefore respectfully requested.

The invention process essentially entails five steps.

They are:

- (a) growing a culture of cells of algae microorganism photoheterotrophically in a Tris-acetate-phosphate medium under white fluorescence illumination conditions to accumulate an endogenous substrate;
- (b) depleting a nutrient selected from the group consisting of sulfur, iron, and/or manganese from the medium in the presence of DCMU¹ by suspending said culture of cells in the absence of said nutrient and sealing the culture of cells of algae microorganism from atmospheric oxygen until conditions become anaerobic;

¹ 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU)
See Plant Biology Vol. 95, Issue 15, 9009-9013, July 21, 1998.

- (c) measuring the rate of cellular oxidative respiration in $\text{m mol O}_2 (\text{mol Chl})^{-1} \text{s}^{-1}$ of a sample of cells of said suspended of algae microorganism from step (b) in the dark until it is constant or about $13 \text{ m mol O}_2 (\text{mol chl})^{-1} \text{s}^{-1}$;
- (d) measuring the rate of O_2 evolution of a sample of the algae microorganism from step (c) under light of saturating intensity of yellow actinic excitation at about $1,300 \mu\text{m photons m}^{-2} \text{s}^{-2}$;
- (e) inducing reversible hydrogenase through photosynthesis by controlling the light saturated rate of oxygen production from the culture of cells of algae microorganism of step (b) so that it is equal to or less than a the constant or $13 \mu \text{ mol O}_2 (\text{mol Chl})^{-1} \text{s}^{-1}$ rate of cellular oxidative respiration using saturating blue actinic excitation at $250 \mu \text{ mol photons m}^{-2} \text{s}^{-1}$ at 700 nm to generate an evolved gas that includes hydrogen.

Summarizing the essentials of the process, applicants would again relate that its process continuously produces hydrogen by inducing reversible hydrogenase in a manner that provides activity of photosynthesis from a light saturated rate of oxygen production P is equal to or less than the rate of cellular respiration of algae microorganism (as shown by the R curve in FIG. 1).

This is significant since it is well known in the prior art for about 60 years that algae will not produce hydrogen gas when oxygen is present because the hydrogenase enzyme that releases hydrogen is not synthesized and is not stable when oxygen is present, wherein the normal plant/algae photosynthetic process splits water and produces oxygen as a by-product, and wherein to get algae to induce the hydrogenase enzyme it has been necessary to use physical (i.e., inert gas bubbling) or chemical (addition of strong reducing agents or biochemical, oxygen-scrubbing systems) means to get rid of the oxygen.

Applicants have resolved this problem by its discovery of a metabolic switch, whereby removing sulfate from the medium of healthy growing algae rapidly decreases the innate ability of the algae to split water and produce oxygen to only about 10% of their normal ability over a 15 to 30 hour period of time. In this sulfate removal process, applicants have further discovered that algae respiration can take up oxygen at about the level or a little greater (rate) than the cells can produce oxygen (at the lower level of production ability) under sulfur-deprived conditions, and that the culture under these conditions will metabolize all the remaining oxygen in a sealed-off culture medium, and the system will rapidly become anaerobic. Hydrogen production continues under these conditions because the hydrogenase enzyme induced in the cells under illumination is stable under controlled oxygen conditions while cell respiration necessary for photosynthesis continues.

Claims 1-3, 5-8 and 10 were rejected as being unpatentable over U.S. Patent 4,442,211 and U.S. Patent 4,010,076 taken with Wykoff et al. under 35 USC 103(a).

Applicants traverse this rejection and request reconsideration for reasons hereinafter provided.

Greenbaum '211 only discloses producing H₂ and O₂ by use of algae in light comprising:

1) subjecting algae in an aqueous phase to light in an environment free of CO₂ and atmospheric O₂ to produce H₂ and O₂ by the action of the light-stimulated algae in splitting water molecules during a first period of time of sufficient duration to produce a physiological stress on said algae;

2) culturing the algae in culture medium in an aerobic atmosphere during a second period of time sufficient to remove the physiological stress; and

3) subjecting the algae in an aqueous phase to light in an environment free of CO₂ and

atmospheric O₂ during a third period of time at an enhanced rate of production of H₂ and O₂ compared to that occurring during the first time period of step 1).

Greenbaum '211 lacks applicants' step (b) of depleting sulfur in the presence of DCMU and sealing off the culture cells until the culture becomes substantially anaerobic.

Further, Greenbaum '211 clearly lacks applicants' steps (c),(d) and (e) which require measuring cellular respiration in the dark, incubating in light of saturating intensity of yellow actinic excitation and measuring the light-saturated rate of O₂ evolution, and inducing reversible hydrogenase through photosynthesis by controlling the light saturated rate of oxygen production so that it is equal to or less than the rate of cellular respiration.

Weetall '076 merely disclose continuous photometabolic production of a useful product, comprising immobilizing whole cells of a photometabolically active organism on a medium to form a stabilized composite, placing the composite within a reactor having at least one light transmitting wall, and, in the presence of light being transmitted through the wall, continuously passing into the reactor a substance capable of being photometabolized by the cells under conditions sufficient to assure the production of a useful product.

Although blue-green algae may be used in the biophotolysis of water by oxidizing the water and reducing NADP to NADPH, it is manifestly clear that the combination of Weetall '076 with Greenbaum '211 fails to suggest or teach applicants' steps (b), (c), (d) and (e). Depletion of the sulfur nutrient and incubating the culture in light to induce reversible hydrogenase to provide activity of photosynthesis from a light saturated rate of oxygen production, equal to or less than the rate of cellular respiration cannot even be remotely inferred from the combination.

The deficiencies described and discussed above in reference to the combination of Greenbaum '211 and Weetall '076 are not compensated for by any teachings in the secondary reference of Wykoff et al.

Wykoff et al. only disclose the extent to which the light-saturated rate of photosynthetic O₂ evolution declines in *Chlamydomonas reinhardtii* upon P and S starvation. It makes no reference to or acknowledgement of, the prior art problem of not being able to sustain hydrogen production due to deactivation of hydrogenase in the presence of oxygen during photosynthetic hydrogen production. Neither does Wykoff et al. provide any solution to this problem.

Consequently, even if Wykoff et al. were combined with Greenbaum '211 and Wetall '076, applicants' process claims as presently amended could not possibly result from this combination.

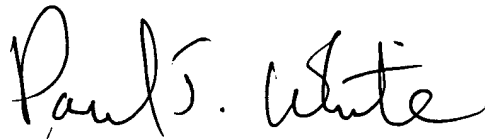
Neither would applicants' process be rendered obvious. This is so because no reference alone or in combination either acknowledge or resolve the problem of providing sustained production of hydrogen by avoiding deactivation of hydrogenase in presence of oxygen by controlling the light saturated rate of oxygen production so that it is equal to or less than the rate of cellular respiration.

Even if the Wykoff et al. teachings of the extent to which the light saturated rate of photosynthetic O₂ evolution declines in *Chlamydomonas reinhardtii* upon P and S starvation were substituted into the processes of the primary references of Greenbaum '211 and Wetall '076, such a substitution would be inadequate without hindsight reference to applicants' invention, to provide a skilled person in the art with means for sustaining production of hydrogen by avoiding deactivation of hydrogenase in the presence of oxygen, as required by applicants' claims.

Applicants take note of the objections raised to claim 6, the rejection of claims 1-3, 5-8 and 10 under the second paragraph of 35 USC §112 on allegations of indefiniteness, and the rejection of claims 1-3, 5-8 and 10 under the first paragraph of 35 USC §112 on allegations of new matter; however, in view of the amendments made to the claims, these objections and rejections are believed no longer applicable.

In view of the foregoing amendments, remarks and arguments, it is believed that the application is now in condition for allowance and early notification of the same is earnestly solicited.

Respectfully submitted,

A handwritten signature in cursive script that reads "Paul J. White". The signature is written in dark ink and is positioned above a horizontal line.

Dated: October 31, 2003.

Paul J. White
Attorney for Applicants
Registration No. 30,436

NATIONAL RENEWABLE ENERGY LABORATORY
1617 Cole Boulevard
Golden, Colorado 80401-3393
Telephone: (303) 384-7575
Facsimile: (303) 384-7499